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## Some factors influencing microbial growth on soil animal faeces

### I. Bacterial and fungal growth on particulate oak leaf litter

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With 5 figures

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#### 1. Introduction

The comminution of plant material by soil animal feeding destroys leaf structure and increases the surface area available for microbial colonisation, and has been considered one of the main processes by which soil animals enhance microbial activity during litter decomposition (VAN DER DRIFT & WITKAMP 1960). GROSSBARD, however, commented on the persistence of oribatid faeces in the soil, and NICHOLSON et al. (1966) found no significant difference in the decay rates of *Glomeris* faeces and their parent leaf litter after one year. The results of these experiments appear contrary and the overall effects of soil animal feeding on the rate of leaf litter decomposition have yet to be established.

Increased surface area, nevertheless, has long been known as a factor enhancing bacterial growth (ZOBELL 1943) and this may account for the large numbers of bacteria found on freshly produced soil animal faeces (VAN DER DRIFT & WITKAMP 1960, and REYES & TIEDJE 1976 & HANLON 1978). There may also be other factors arising from the digestive processes within soil animal intestines which may account for this increase in bacterial numbers.

Examinations, however, of fungal and bacterial colonisation of artificial substrates by PARR & NORMAN (1964), PARR et al. (1967) and GRIFFIN (1963a, b) suggested that a reduction in particle size below a certain level inhibited fungal growth, but had no effect on bacterial growth. The reduction in particle size of a substrate not only increases surface area, but also decreases the pore space between particles. The crucial requirements for microbial growth were considered by PARR & NORMAN to be linear surface area for bacteria and available pore space for fungi. Microbial colonisation of faeces may therefore be influenced by the particle sizes within faecal pellets.

The aim of this investigation was to measure fungal and bacterial activity on leaf litter of different particle sizes, to determine the factors influencing microbial colonisation of the particulate material present in soil animal faeces.

## 2. Materials and methods

### 2.1. Preparation and inoculation of leaf litter

Samples of oven dried fresh oak (*Quercus robur* L.) leaf litter were ground to different particle sizes in an analytical mill, then shaken through a series of Endicott sieves, producing seven particle size ranges of diameters 1–2 mm, 0.5–1 mm, 0.3–0.5 mm, 0.2–0.3 mm, 0.11–0.2 mm, 0.11 to 0.053 mm and less than 0.053 mm.

Samples (0.1 g) of ground litter were soaked for 24 hours in 15 mls of sterile water to allow reconstitution of the water lost during the drying. The suspension of litter was then washed with sterile water into a 45 mm diameter Millipore filter apparatus connected to a vacuum pump. The litter was then drawn onto a glass fibre filter (Whatmans GF/A 55 mm circles) to form a deposit 45 mm in diameter on the filter. After deposition the sample was flooded with 2.0 cc of 0.8 mg cc<sup>-1</sup> Crystamycin solution for 5 minutes to inhibit bacterial growth (WEBSTER & DAVEY 1975). After this period the excess water was drawn off with a vacuum pump but leaving the material sufficiently moist to sustain microbial growth.

The filters containing the litter were folded to enclose the sample and placed in a 15 ml Warburg reaction flask and sealed with aluminium foil. The samples were then sterilised in the flasks by gamma radiation, receiving a dosage of  $2.5 \times 10^6$  Rads emitted from a Co<sup>60</sup> radioactive source. Sterile water (0.2 cc) was pipetted into the centre well of the flask to maintain moisture levels, and the samples then respired in Warburg respirometers at 25 °C, using oxygen consumption as the measurement of fungal activity. Samples (0.5 cc) of 0.1 N KOH were added to the flask side arms and renewed daily before readings. Alkali was also left in the flasks between readings to prevent the accumulation of CO<sub>2</sub> which might inhibit microbial growth (MACFADYEN 1973).

This procedure was repeated using the same quantity of litter deposited on different filter surface areas to examine the effect of compaction of the ground litter on microbial growth. Two 0.5 g samples of litter were prepared as mentioned previously and deposited on 2 filters and both filters respired in one flask, and 0.2 g samples were deposited on one filter which was then cut in half and each half respired separately. This compacted the litter fragments on the smaller filter areas by producing thicker layers of particulate material. These, together with the first sample, gave 0.1 g of oak litter deposited on 3 filter areas, i.e. 1075 mm<sup>2</sup> (half a filter), 2150 mm<sup>2</sup> (1 filter) and 4300 mm<sup>2</sup> (2 filters).

After sterilisation the samples were inoculated by placing small plugs of comminuted oak litter, on which the fungus *Coriolus versicolor* (L. ex Fr.) had been cultured, onto the filters. Five replicate samples of each of the 7 particle size ranges deposited on the 3 filter areas, were prepared and respired daily over a 2 hour period for 25 days.

The above procedure was repeated to measure bacterial activity on ground litter, with the following adjustments. The leaf litter, after deposition on the glass fibre filter, was flooded with a solution of 100 µg cc<sup>-1</sup> Nystatin and Cycloheximide (B. D. H. Chemicals Ltd.) to inhibit fungal growth (WILLIAMS & DAVIES 1968). The bacterial inoculum was prepared from an unidentified coccoid bacterium, isolated on 2% peptone agar, from the faeces of the Isopod *Oniscus asellus* L. fed on partially decayed leaf litter. Several small colonies of bacteria were aseptically removed and dispersed in 10 ml sterile water in a M. S. E. blender for 5 mins, and 1 cc of the resultant suspension was diluted to 50 mls in a volumetric flask. 0.1 cc of the dilution was then pipetted onto the litter samples, which had been sterilized by gamma radiation. The inoculum was found to contain approximately  $3 \times 10^8$  bacterial cells (enumerated by a direct count technique) (HANSEN et al. 1974). Bacterial respiration was examined on 5 particle size ranges (1–2 mm, 0.5–1.0 mm, 0.3–0.5 mm, 0.2–0.3 mm and 0.053–0.11 mm) deposited on the 3 filter areas, and 4 replicates were prepared for each treatment.

The experiment was repeated using a mixed inoculum of *C. versicolor* and bacteria as previously described and the same particle sizes and filter areas used for bacteria. No antibiotics were used in this experiment. The results were statistically analysed using the Student Newman Keuls multiple comparison test (SOKAL & ROHLF 1969) at a significance level of  $P = 0.05$ .

## 3. Results

### 3.1. Fungal respiration

Fig. 1 shows the respiration patterns of *C. versicolor* grown on 4 of the particle size ranges deposited on a filter area of 4300 mm<sup>2</sup> over a period of 25 days. A short initial lag phase

Fig. 1. Rates of oxygen consumption by *Coriolus versicolor* growing on 4 particle size ranges of leaf litter deposited on a glass fibre filter of surface area 4300 mm<sup>2</sup>. Mean fungal respiratory rates ( $\pm 95\%$  confidence limits,  $n = 5$ ) are shown for cultures containing litter of particle sizes  $> 0.053$  mm (—●—), 0.1–0.2 mm (—○—), 0.3–0.5 mm (—▼—) and 1.0–2.0 mm (—■—).

Fig. 2. Maximum oxygen consumption levels by *Coriolus versicolor* growing on 7 particle size ranges deposited on 3 different filter surface areas. Mean fungal respiratory rates ( $\pm 95\%$  confidence limits,  $n = 5$ ) are shown for cultures grown on surface areas, 4300 mm<sup>2</sup> (—■—), 2150 mm<sup>2</sup> (—▼—) and 1075 mm<sup>2</sup> (—●—).

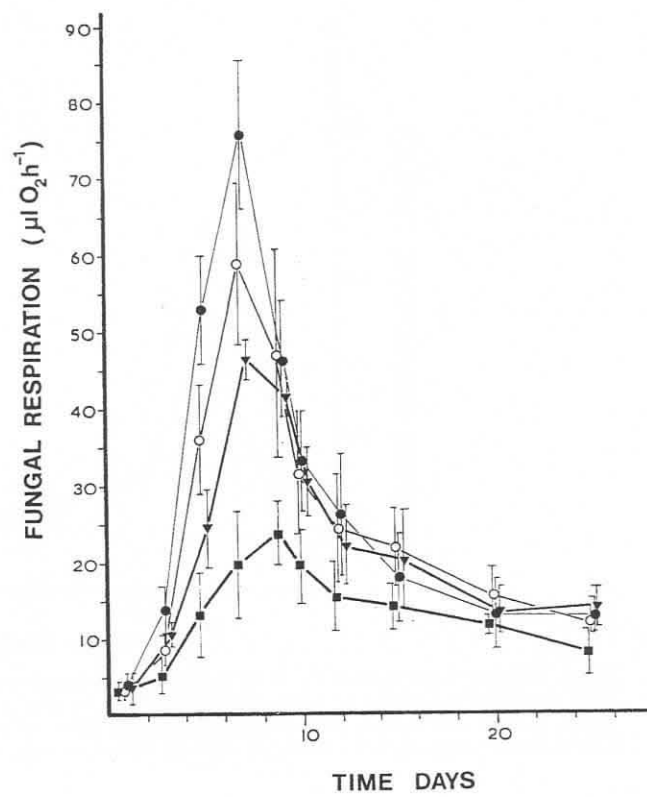


Fig. 1

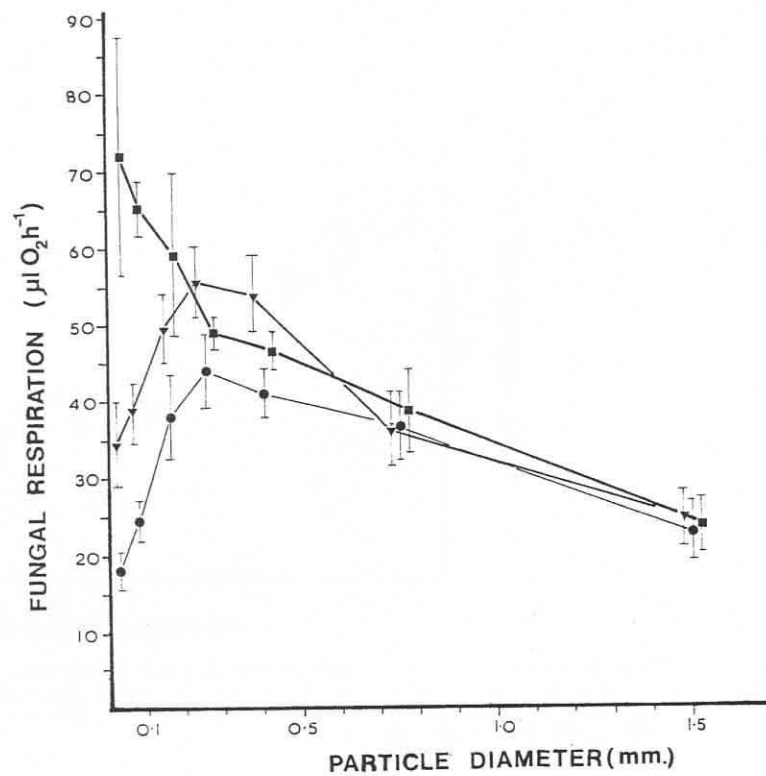


Fig. 2

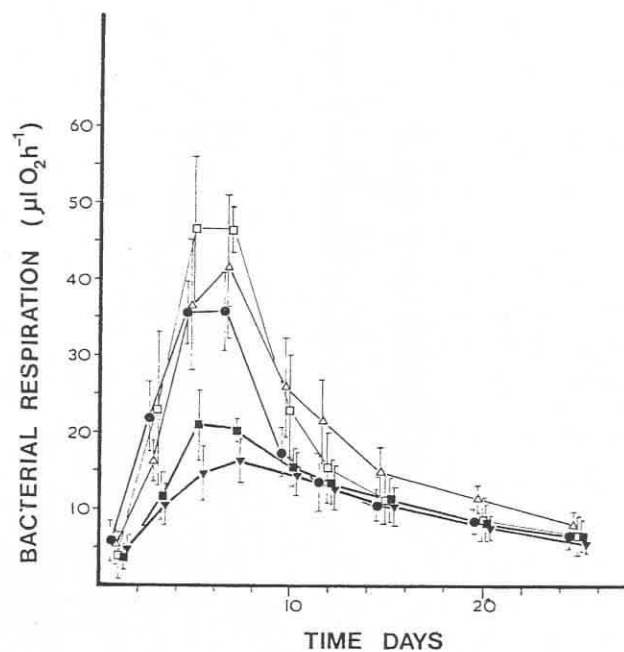


Fig. 3. Oxygen consumption rates of bacterial cultures grown on 5 particle size ranges of oak leaf litter deposited  $\mu$ n filters of area 4300 mm<sup>2</sup>. Mean bacterial respiratory rates ( $\pm$  95 % confidence limits,  $n = 4$ ) are shown for cultures containing litter of particle sizes 0.053–0.1 mm ( $\square$ ), 0.2–0.3 mm ( $\triangle$ ), 0.3–0.5 mm ( $\bullet$ ), 0.5–1.0 ( $\blacksquare$ ) and 1.0–2.0 mm ( $\blacktriangledown$ ).

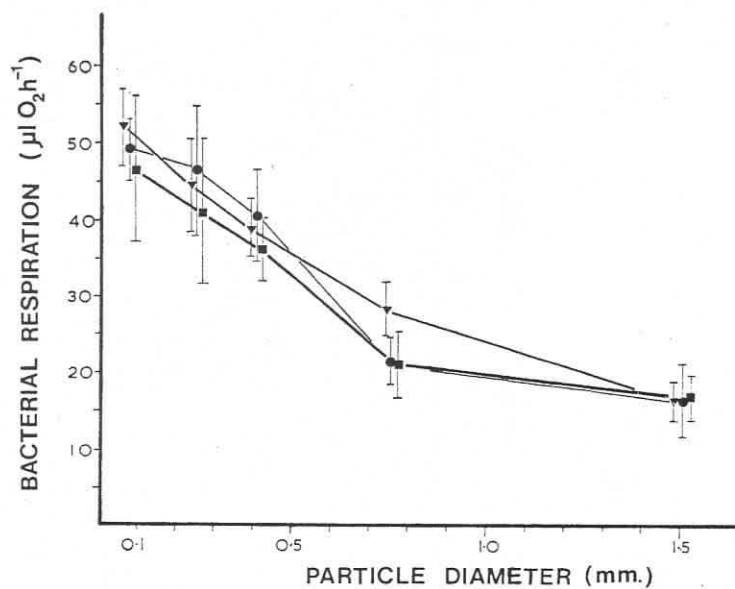


Fig. 4. Maximum oxygen consumption rates of bacterial cultures growing on 5 particle size ranges of oak leaf litter deposited on 3 different filter surface areas. Mean bacterial respiration rates ( $\pm$  95 % confidence limits,  $n = 4$ ) are shown for cultures grown on surface areas, 4300 mm<sup>2</sup> ( $\blacksquare$ ), 2150 mm<sup>2</sup> ( $\blacktriangledown$ ) and 1075 mm<sup>2</sup> ( $\bullet$ ).

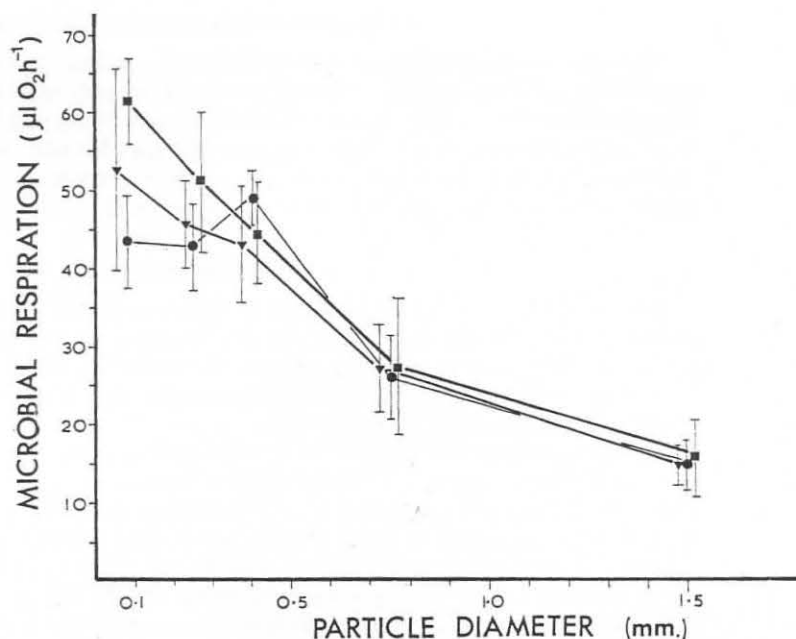


Fig. 5. Maximum oxygen consumption rates for mixed cultures of *Coriolus versicolor* growing on 5 particle size ranges of oak leaf litter deposited on 3 filter surface areas. Mean microbial respiratory (rates)  $\pm$  95 % confidence limits,  $n = 4$  are shown for cultures grown on surface areas, 4300 mm<sup>2</sup> (—■—), 2150 mm<sup>2</sup> (—▼—) and 1075 mm<sup>2</sup> (—●—).

was present over the first few days, which was followed by a rapid increase in O<sub>2</sub> consumption to maximum values after 8 to 10 days, after which respiration rapidly declined. Maximum O<sub>2</sub> consumption here was greatest on the smallest litter particle sizes. Fungal respiration, however, measured from litter samples consisting of the smaller particle sizes deposited on filter areas of 1075 mm<sup>2</sup> and 2150 mm<sup>2</sup>, was much less than that recorded for the largest filter area. The results for maximum fungal O<sub>2</sub> consumption on the 7 particle sizes deposited on the 3 surface areas are summarised in Fig. 2. O<sub>2</sub> consumption on the largest filter area (4300 mm<sup>2</sup>) shows an inverse relationship with particle size. On other filters, however, fungal respiration shows an initial increase with reduction in particle size, which then begins to decrease as the particles become smaller, the decline being greater on the smallest filter area (1075 mm<sup>2</sup>). Fungal O<sub>2</sub> consumption showed no significant differences (at  $P = 0.05$ ) between filter areas in the large particle size ranges (1.0–2.0 to 0.2–0.3 mm) but did show a significant decline ( $P < 0.05$ ) in respiration on the 2 smaller filter areas containing the remaining particle sizes (0.11–0.2, 0.053–0.11, and  $< 0.053$  mm).

### 3.2. Bacterial respiration

The O<sub>2</sub> consumption rates for bacteria grown on 5 particle sizes deposited on a filter area of 4300 mm<sup>2</sup> are shown in Fig. 3. Bacterial respiration increased rapidly to maximum at approximately 6 days after inoculation, then declined rapidly to a lower level. Bacterial O<sub>2</sub> consumption on the remaining filters showed similar patterns. These effects are summarised in Fig. 4 and show the maximum O<sub>2</sub> consumption levels for all 5 particle size ranges deposited on the 3 filter areas. In each case bacterial respiration shows an inverse relationship with particle diameter. There were no significant differences (at  $P = 0.05$ ) among bacterial respiration rates on the different filter areas, with the exception of 1 size range (0.5–1.0 mm) on the filter area of 2150 mm<sup>2</sup>, where O<sub>2</sub> consumption is significantly greater ( $P < 0.05$ ) than that of the other filters. The reasons for this are unknown, but may be the result of contamination.

### 3.3. Mixed microbial respiration

Microbial respiration from samples containing a mixed inoculum showed a rapid increase followed by a decline similar to that measured for bacteria over the 25 day period. Maximum fungal and bacterial respiration rates for all the size ranges are summarised in Fig. 5. Maximum microbial oxygen consumption again shows an inverse relationship with particle diameter. Compaction of the samples had no effect on maximum respiration values as oxygen consumption rates were not statistically different at  $P = 0.05$ .

### 4. Discussion

The experiments of VAN DER DRIFT & WITKAMP (1960) have been widely interpreted to show that the main effect of leaf litter comminution by soil animals is the increase in the surface area exposed to microbial attack. The results reported here, however, suggest that the physical effects of soil animal feeding activities may be more complex than this and may either enhance or inhibit fungal growth.

Fungal and bacterial respiration here showed a progressive increase, with a reduction in particle size on ground leaf litter samples deposited in a thin layer covering a large surface area. Fungal growth, however, was restricted in compacted samples containing finely ground litter deposited in much thicker layers. The compaction of the leaf litter did not influence bacterial respiration in the particle size ranges examined here, and had only a small depressive effect in mixed cultures grown on the smallest particle size. Examination of the litter samples by a scanning electron microscope (HANLON 1978) showed that the fungal mycelium was almost entirely restricted to the surface layers of compacted samples consisting of small particles (0.2–0.11, 0.11–0.053 and  $> 0.053$  mm). Micrographs, however, of the larger litter fragments (over 0.3 mm diameter) showed an extensive mycelium ramifying the pore space between particles.

Similar observations have been made by PARR & NORMAN (1964) who suggested that the size of the pore spaces between particles was important for fungal colonisation of a resource. ADU & OADES (1978a, b) also suggested that pore size in soil aggregates influenced microbial colonisation of the soil surface area and was important in rendering the proportion of material available to microbial attack. Nevertheless many fungi can penetrate woody tissues as a result of extracellular digestion and certain aspects of fungal colonisation may not be reliant on available pore space. The phenomena here may therefore relate only to the initial colonisation phase of fungi, in which the fungi try to occupy as much of the resource area as quickly as possible.

The results here also suggest that compacted samples of finely comminuted litter tend to favour bacterial colonisation and fungal growth favoured on larger particle sizes. The proportions of bacteria and fungi growing on comminuted plant material may therefore be determined by particle size. An examination of plant material in soil macroarthropod faeces (HANLON 1978) showed a wide variety of particle sizes ranging from 0.5 mm to less than 0.01 mm in diameter. The arrangement of these particles within faeces and the formation of aggregates in the soil by faecal pellets may be important in determining the extent of bacterial and fungal growth in the soil.

There are, however, many other factors influencing microbial growth on soil animal faeces, such as the enrichment of the resource by gut secretions, the partial digestion of plant litter and a change in pH of the litter during passage through the gut (VAN DER DRIFT & WITKAMP 1960), which will have to be incorporated into this experimental framework for a more comprehensive interpretation of microbial growth on soil animal faeces.

### 5. Summary · Zusammenfassung

The growth of bacteria, isolated from soil animal faeces, and of the fungus *Coriolus versicolor* in separate and mixed cultures, was examined on mechanically ground oak leaf litter which had been sieved into a series of particle sizes ranging from 1.0–2.0, —  $> 0.053$  mm. The litter was deposited in layers of varying thickness to investigate the effect of compaction on microbial growth.

Maximum oxygen consumption levels of bacteria and fungi showed an inverse relationship with particle size in litter samples deposited in the thinnest layers. Fungal growth was reduced in compacted samples consisting of small particles ( $> 0.2$  mm), as a result of a reduction in pore space. Compaction, however, did not reduce bacterial growth on the particle size ranges examined. The results are discussed in relation to the influence of leaf litter comminution by soil animals on microbial growth on particulate material in faeces.

#### Einige Faktoren, die das Mikробenwachstum auf Bodentier-Faeces beeinflussen.

##### I. Bakterien- und Pilzwachstum auf zerkleinerter Eichenblattstreu

Das Wachstum der aus dem Kot von Bodentieren isolierten Bakterien und des Pilzes *Coriolus versicolor* in gesonderten und gemischten Kulturen wurde auf mechanisch zerkleinerter, in einer Reihe von Teilchengrößen von 1,0–2,0 und  $> 0,053$  mm gesiebter Eichenlaubstreu, untersucht. Die Streu wurde in Schichten von verschiedener Dicke abgesetzt, um die Einwirkung der Lagerungsdichte auf das Wachstum der Mikroben zu prüfen. Die maximale Höhe des Sauerstoffverbrauchs durch Bakterien und Pilze zeigte einen negativen Zusammenhang mit der Teilchengröße der Laubstreu in den dünnsten Schichten. Das Mycelwachstum war in dickschichtigen Proben, die aus kleinen Teilchen ( $> 0,2$  mm) bestehen, herabgesetzt, was auf einen geringeren Porenraum zurückzuführen ist. Die Lagerungsdichte hat den Wuchs der Bakterienkolonien auf den von uns untersuchten Teilchengrößen der Streu nicht gehemmt. Diese Ergebnisse werden in Hinsicht auf die Zerkleinerung der Streu durch Bodenorganismen und auf das Wachstum der Mikroben auf zerkleinerten organischen Stoffen im Kot erörtert.

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